



Atty Dkt. No.:CLON-060  
USSN:09/960,716

<b>DECLARATION OF GRIGORIY TCHAGA UNDER 37 C.F.R. § 1.132</b>  Address to: Commissioner for Patents P.O. Box 1450 Alexandria VA 22313-1450	Attorney Docket Confirmation No.	CLON-060 4277
	First Named Inventor	Grigoriy Tchaga
	Application Number	09/960,716
	Filing Date	September 21, 2001
	Group Art Unit	1641
	Examiner Name	Ann Y Lam
	Title: <b>HIGHLY SENSITIVE PROTEOMIC ANALYSIS METHODS, AND KITS AND SYSTEMS FOR PRACTICING THE SAME</b>	

Dear Sir:

1. I, Grigoriy Tchaga, declare and say I am a resident of the State of California. My residence address is 5322 Yarmouth, Newark, CA 94560.
2. I hold a Ph.D., which I received from Uppsala University, Uppsala, Sweden in 1994. I am skilled in the fields of Biochemistry and Molecular Biology. I am a co-inventor of the invention claims of the above-referenced patent application.
3. I have reviewed the relevant portions of the current Office Action mailed June 13, 2006, in the above-referenced application. I understand that claims of the above-referenced patent application are rejected under 35 USC §103(a) on the grounds that they are unpatentable over the combined teachings of the following references:
  - Margherita et al. (4,111,656) in view of Zarling et al. (5,674,698) and further in view of Kartel et al. (Chemosphere, vol. 38, pp. 2591-2596);
  - Margherita et al. (4,111,656) in view of Zarling et al. (5,674,698) and further in view of Kartel et al. (Chemosphere, vol. 38, pp. 2591-2596), Schoemaker et al. (4,837,167) and Pronovost et al. (5,773,234);
  - Margherita et al. (4,111,656) in view of Zarling et al. (5,674,698) and further in view of Kartel et al. (Chemosphere, vol. 38, pp. 2591-2596) and Wohlstadter et al. (6,207,369);

- Margherita et al. (4,111,656) in view of Zarling et al. (5,674,698) and further in view of Kartel et al. (Chemosphere, vol. 38, pp. 2591-2596), Schoemaker et al. (4,837,167), Pronovost et al. (5,773,234) and Wohlstadter et al. (6,207,369);
  - Velander et al. (5,328,603) in view of Zarling et al. (5,674,698) and further in view of Kartel et al. (Chemosphere, vol. 38, pp. 2591-2596); and
  - Velander et al. (5,328,603) in view of Zarling et al. (5,674,698) and further in view of Kartel et al. (Chemosphere, vol. 38, pp. 2591-2596), Schoemaker et al. (4,837,167), Pronovost et al. (5,773,234) and Wohlstadter et al. (6,207,369).
4. This Declaration provides further evidence of the patentability of the claimed invention. Specifically, this Declaration provides evidence of unexpected results obtained by employing the methods of the claimed invention. Specifically, the data shown in Exhibit A demonstrate that the inclusion of a metal ion chelating polysaccharide as claimed (i.e., pectin) significantly reduces background in an array-based analyte detection assay.
5. The following experiments were conducted by me or under my direction.
6. Exhibit A shows the results of two array-based analyte detection assays performed under standard assay conditions (i.e., sample contains no metal chelating polysaccharide; right panel) and under conditions as claimed in the subject invention (sample contains 0.2% pectin, a metal chelating polysaccharide; left panel). The arrays employed are identical arrays of antibodies specific for protein analytes. The sample contacted to both arrays is a HeLa cell protein extract directly labeled with the fluorescent dye Cy3. The data in Exhibit A clearly demonstrate that the background fluorescence on the array contacted with the sample containing pectin (left panel) is far lower than the background fluorescence on the array contacted with the sample without pectin (right panel). The average fluorescent background on the

array contacted to the sample containing pectin (left panel) was calculated as approximately 200 relative fluorescent units (RFUs) whereas the average fluorescent background on the array contacted to the sample lacking pectin (right panel) was calculated as approximately 35,000 RFUs. Therefore, the decrease in background in this experiment is approximately 175-fold.

7. The data provided support the assertion that the inclusion of a metal ion chelating polysaccharide provides an unexpected reduction in the background fluorescence in array based analyte detection assays. In view of these unexpected results, the claimed invention is not obvious over the prior art relied upon to reject the claims as obvious in the current Office Action.
8. I, Grigoriy Tchaga, hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such will false statements may jeopardize the validity of the application or any patent issuing thereon.

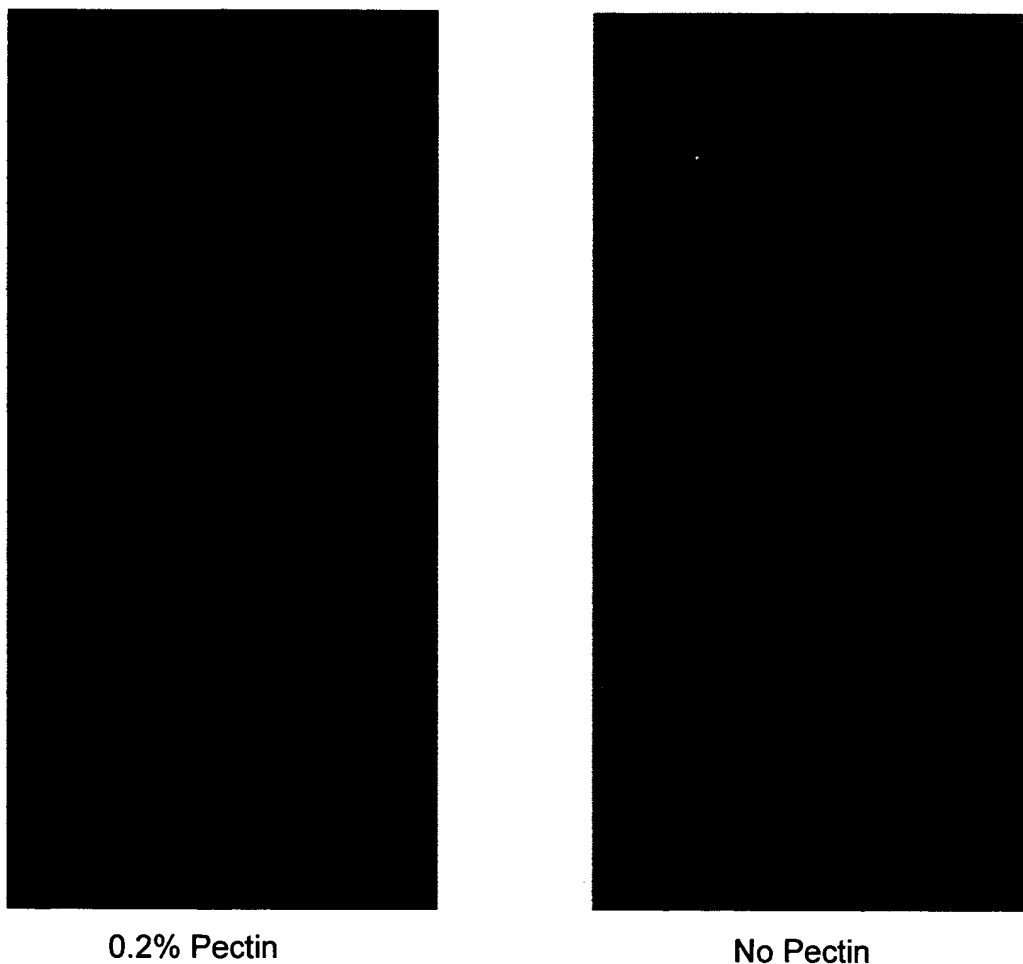
9/28/2006  
Date

Grigoriy S. Tchaga  
Grigoriy Tchaga, Ph.D.

Attachments: Exhibit A, 1 page

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## EXHIBIT A



10  $\mu$ g of protein from a HeLa cell extract was directly labeled with Cy3 and contacted to antibody arrays on glass slides in the presence (left panel) and absence (left panel) of Pectin. After incubation, the arrays were washed and Cy3 fluorescence associated with the array was measured. As is clear from this figure, Pectin significantly reduced the background fluorescence (from 35,000 RLU to 200 RLU) of the array, allowing enhanced detection sensitivity of antibody/analyte interactions (i.e., more antibody spots binding analyte are seen in the pectin-containing sample).